Data Sheet (21.12.2016)

T4 DNA Ligase

Source: *E. coli* lambda lysogen NM 989

Cat.-No.	Amount	Conc.
mi-E0149S	400 Weiss units	2,5 WU/µl
mi-E0149L	5x 400 Weiss units	2,5 WU/µl

Storage conditions: - 20 ± 5°C
For research use only! Only for* in vitro *use!

Notes:

- One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of ^{32}P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37 °C.
- T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 200 mM.
- Ligation of blunt-ended and single-base pair overhang fragments requires about 50 times as much enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Blunt-end ligation may be enhanced by addition of PEG 4000 (10 % w/v final concentration) or hexamine chloride, or by reducing the ATP concentration to 50 µM.
- To dilute T4 DNA Ligase that will subsequently be stored at –20 °C, 50 % glycerol storage buffer should be used; to dilute for immediate use, 1x T4 DNA Ligase reaction buffer can be used.
- One Cohesive-End Ligation Unit (CEU) is defined as the amount of enzyme required to give 50 % ligation of Hind III fragments of λ DNA (5’ DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16 °C in 1x T4 DNA Ligase Reaction Buffer.
- One Weiss unit is equivalent to approx. 67 CEU.

Description: T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5’-phosphate and 3'-hydroxyl termini in duplex DNA or RNA.

Reaction conditions: 50 mM Tris-HCl (pH 7.8 at 25°C), 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP and 2,5µg/ml BSA, 0,1-1 Weiss Units of T4 DNA Ligase and DNA (100-200 ng vector DNA).
Optimal ligation occurs at 16 °C.

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50 % glycerol.

Heat inactivation: T4 DNA Ligase can be inactivated by incubation at 65 °C for 10 minutes.